Talanta 178 (2018) 299–307

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Preparation and evaluation of magnetic molecularly imprinted polymers for the specific enrichment of phloridzin

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ARTICLE INFO

Keywords:
Magnetic molecularly imprinted polymers
Phloridzin
Leaves of *Malus doumeri* (Bois) A. Chev
Rats' plasma

ABSTRACT

In present study, magnetic molecularly imprinted polymers (MMIPs) were successfully prepared for specific recognition and selective enrichment of phloridzin from the leaves of *Malus doumeri* (Bois) A. Chev and rats' plasma. The magnetic Fe_3O_4 were prepared by the solvothermal reaction method and followed by the modification of TEOS and functionalization with APTES. Using functionalized Fe_3O_4 particles as the magnetic cores, phloridzin as template, ethylene glycol dimethacrylate (EGDMA) as cross-linker and 2,2-azobisisobutyonnitrile (AIBN) as initiator, the MMIPs were prepared through APTES to associate the template on the surface of the magnetic substrate. The structural features and morphological characterizations of MMIPs were performed by FT-IR, SEM, TEM, XRD, TGA and VSM. The adsorption experiments revealed that the MMIPs presented high selective recognition property to phloridzin. The selectivity experiment indicated that the adsorption capacity and selectivity of polymers to phloridzin was higher than that of baicalin and 2,3,5,4′-ttrahydroxy stilbene-2-O-β-D-glucoside. Furthermore, the MMIPs were employed as adsorbents for extraction and enrichment of phloridzin from the leaves of *M. doumeri* and rats' plasma. The recoveries of phloridzin in the leaves of *M. doumeri* ranged from 81.45% to 90.27%. The maximum concentration (C_{max}) of phloridzin in rats' plasma was detected as 12.19 ± 0.84 μg/mL at about 15 min after oral administration of phloridzin (200 mg/kg). These results demonstrate that the prepared MMIPs are suitable for the selective adsorption of phloridzin from complex samples such as natural medical plants and biological samples.

1. Introduction

The molecular imprinting technique (MIT) is a promising and facile separation and enrichment technique for molecular recognition with high sensibility and selectivity [1]. Nowadays, MIT has widely applied in solid phase extraction [2], solid phase microextraction [3], chromatography [4], biomimetic sensors [5] and for residue analysis in food producing animals [6,7]. The molecularly imprinted polymers (MIPs) which prepared by using MIT own a specific memory function and possess the performances of high selectivity and recognition ability to the target compounds [8]. Thus, MIPs have potential to be the effective adsorption materials for selective separation and recognition of template molecules during sample pretreatment [9,10]. In the preparation process of MIPs, bulk polymerization is the conventional synthetic method. However, the MIPs prepared by this method need to be crushed and sieved, and usually in irregular shape [11]. Moreover, the separation of MIPs from sample solution needs high speed centrifugation. It is gratifying that magnetic molecularly imprinted polymers (MMIPs), which prepared by using surface imprinting technique, can overcome the problems brought by MIPs which prepared by bulk polymerization [12]. The most of the binding sites on the surface of particles make the MMIPs easily to bind and remove of the templates. Moreover, with an external magnet, MMIPs can be easily separated from the sample solution [13,14]. Therefore, in recent years, MMIPs have been widely used as selective adsorbents to extract various molecules from complex matrices.

Abbreviations: MMIPs, magnetic molecularly imprinted polymers; MNIPs, magnetic non-molecularly imprinted polymers; HPLC, high-performance liquid chromatography; EGDMA, ethylene glycol dimethyl acrylate; AIBN, 2,2-azobisisobutyonnitrile; TEOS, tetraethoxysilane; APTES, 3-ammonia propyl triethoxy silane; CMC-Na, carboxy methyl cellulose sodium; SEM, scanning electron microscopy; TEM, transmission electron microscopy; FT-IR, Fourier transform infrared; XRD, X-ray diffraction; TGA, thermal gravimetric analysis; LOD, limit of detection; LOQ, limit of quantification; RSD, relative standard deviation

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http://dx.doi.org/10.1016/j.talanta.2017.09.058
Received 21 June 2017; Received in revised form 13 September 2017; Accepted 17 September 2017
Available online 20 September 2017
0039-9140/

On the other hand, *Malus doumeri* (Bois) A. Chev, which belongs to genus *Malus* of *Rosaceae* family, is one of the well-known medical plant native to Taiwan and south China [15,16]. The leaves of *M. doumeri* have been commonly consumed as a healthy beverage in folk. It is said that the leaves of *M. doumeri* have been proved to have heat-related hyperactivity, anti-oxidant and anti-proliferative activities and they also used as a tonic to reinforce vital energy [17,18]. Phloridzin, a kind of natural dihydrochalcone, is the main effective component of the leaves of *M. doumeri* [19]. It has various biological activities, such as anti-inflammatory, anti-oxidative, anti-diabetic, anti-tumor activities and can be used as longevity extending agents in foods, beverages, food additives, pharmaceuticals and cosmetics [20–22]. However, till now, the separation and enrichment of phloridzin from the leaves of *M. doumeri* is usually based on column chromatography [23] and high-speed counter-current chromatography coupled online to high performance liquid chromatography (HSCCC-HPLC) [19]. Though column chromatography and HSCCC-HPLC method can enrich and purify phloridzin, they need large solvent consumption and the selectivity of them was poor. Moreover, column chromatography will lead to low recovery and traces losing of effective compounds and HSCCC-HPLC method needs expensive instruments. It is noteworthy that MMIPs can be prepared easily with low cost and they perform high selectivity towards the specific analyte in complex system. They can selectively extract phloridzin from the leaves of *M. doumeri*. Moreover, by using an external magnetic field, MMIPs can easily be separated from a matrix [24]. Therefore, compared with the column chromatography and HSCCC-HPLC method, the MMIPs based extraction method can more rapidly and more effectively to extract phloridzin from the leaves of *M. doumeri* with less solvent consumption.

In addition, it is noteworthy that elucidation of the absorption and metabolism of an active compound in blood and et al. is very important to understand the roles it played in pharmacological and biological processes [25,26]. Therefore, it is necessary to explore the absorption and metabolism of phloridzin in blood. However, few studies about the pharmacokinetic of phloridzin in blood have been reported to date.

In present study, MMIPs with core-shell structure by using Fe_3O_4 particles as magnetic cores, mesoporous polymers as shell and phloridzin as the template were prepared for the first time. The MMIPs were successfully synthesized by using surface imprinting polymerization. The prepared polymers were characterized by Fourier transform infrared (FT-IR) spectroscopy, scanning electron microscopy (SEM) , transmission electron microscope (TEM), thermal gravimetric analysis (TGA), x-ray diffraction (XRD) and vibrating sample magnetometry (VSM). The adsorption and selectivity properties of the MMIPs were also investigated in detail. Finally, the MMIPs were applied to the adsorbent materials of magnetic solid-phase extraction (MSPE) coupled with HPLC for the selective adsorption and determination of phloridzin in the leaves of *M. doumeri* and rats' blood.

2. Experimental

2.1. Chemical and reagents

The leaves of *M. doumeri* were obtained from the local drug store. Phloridzin was separated and purified in our group (purity > 98%). 2,3,5,4′-Tetrahydroxy- stilbene-2-O-β-D-glucoside, baicalin were purchased from PUSH Bio-technology Co., Ltd. (Sichuan, China). Iron(III) chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), sodium acetate andydrous (NaAc), ammonium hydroxide, sodium citrate and anhydrous toluene were obtained from Chengdu Kelong Chemical corporation. Ethyleneglycol dimethacrylate (EGDMA), 2,2′-azobisisobutyronitrile (AIBN), tetraethoxysilane (TEOS) and 3-ammonia propyl triethoxy silane (APTES) were obtained from Aladdin Reagents (Shanghai, China). Deionized water in the experiment was prepared by Aquapro Water Purification System (Chongqing, China). EGDMA were used after vacuum distillation. AIBN was used after recrystallization. Methanol was of chromatographic grade and obtained from Adamas Reagent Co., Ltd (Shanghai, China). All solutions used for HPLC were filtered through a 0.22 μm filter before use.

2.2. Animals

Twenty-seven conscious male Sprague-Dawley rats (200–250 g, the Animal Centre, Chongqing Medical University) were used as subjects for experiments. The rats were divided into two groups (group A, drug group for dosed rat plasma, n = 24; group B, control group for blank rat plasma, n = 3). The animals were acclimatized to their new environment for a minimum of 5 days prior to experiments. Before experiments, food was not given for 14 h and water was available at any time. All experimental procedures were approved by the Institutional Animal ethical Committee of Chongqing University, and were conducted according to the Guide for the Care Use of Laboratory Animal of National Institute of Health (Publication No. 80-23, revised 1996).

2.3. Apparatus

The morphological evaluation was performed by JSM-7600F Field emission scanning electron microscope (JEOL, Japan). The morphological evaluation was performed by transmission electron microscopy (TEM, Zeiss LIBRA 200FEG). The FT-IR spectra of MIPs and NIPs were determined using a Fourier transform infrared spectrometer (IR-Affinity-1, Shimadzu, Japan). The samples were ground with anhydrous KBr and the spectra recorded between 4000 and 500 cm^{-1}. Thermal gravimetric analysis (TGA) was carried out from 25 °C to 900 °C with a heating rate of 10 °C min^{-1} under nitrogen environment by thermal gravimetric analyzer (TGA/DSC1/1600LF, Mettler-Toledo, Switzerland), presenting TGA and derivative thermogravimetry (DTG) data. X-ray diffraction (XRD) measurements were performed on an Ultima IV X-ray diffractometer (Pabakytucal X′ pert powder, Spectris Pte. Ltd, Singapore). The magnetic properties were measured by a vibrating sample magnetometer (Microsense-EZ9, USA).

2.4. Preparation of phloridzin magnetic molecularly imprinted polymers (MMIPs)

2.4.1. Synthesis of Fe_3O_4@SiO_2 particles

The Fe_3O_4 magnetic microspheres were firstly synthesized by the solvothermal reaction method [27]. Typically, 2.7 g $FeCl_3{\cdot}6H_2O$ was dissolved in 80 mL glycol in a 200 mL flask with vigorously stirring. Then, 6.0 g andydrous sodium acetate and 0.8 g sodium citrate were added into the flask. The reaction mixture was refluxed in an oil bath stirring speed of 500 rpm at 150 °C for 1 h. After that, the solutions were transferred to a 100 mL Teflon-lined autoclave and reacted for 6 h in a 200 °C oven, and the black Fe_3O_4 resultants were collected. Subsequently, 600 mg Fe_3O_4 nanoparticles were dispersed into 60 mL isopropanol-ultra-pure solvent (v/v, 5:1) in a 150 mL round flask and ultrasonically treated for 20 min. Then, 10 mL $NH_3{\cdot}H_2O$ and 4 mL TEOS were added dropwise. After reaction for 12 h with constant stirring at room temperature, the brown black resultants Fe_3O_4@SiO_2 were washed with ultra-pure water, and dried under vacuum at 50 °C.

2.4.2. Synthesis of Fe_3O_4@SiO_2 @ NH_2 particles

400 mg of Fe_3O_4@SiO_2 were dispersed in 30 mL of anhydrous toluene in presence of 1 mL of APTES, the dispersion being refluxed during 20 h under mechanical stirring in a nitrogen atmosphere. After that, the products were collected by using an external magnet, rinsed with methanol several times until the supernatant became clearer then dried under vacuum at 50 °C for 24 h.

2.4.3. Preparation of MMIPs

MMIPs was synthesized by surface imprinting polymerization, using phloridzin as template molecule, Fe_3O_4@SiO_2@NH_2 as function